The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL

PROJECT DETAILS	
Project Title	Defining the radiation-induced immune landscape in primary breast cancers for optimal radio-immunotherapy combinations
Short Project Title	Radiation-induced immune landscape in breast cancers

SUPERVISORY TEAM	
Primary Supervisor	Navita Somaiah
Associate Supervisor(s)	Yinyin Yuan, Erik Wennerberg
Secondary Supervisor	Andrew Tutt
IRS Partner	Alan Melcher
DIVISIONAL AFFILIATION	
Primary Division	Radiotherapy and Imaging
Primary Team	Translational Breast Radiobiology
Site	Sutton

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

A fundamental understanding of the molecular and architectural changes wrought in primary breast tumour tissue and microenvironment by therapeutic irradiation is required to inform rational design of clinical trials in this area [1]. Recent evidence suggests a strong cross-talk between DNA damage response (DDR) and immune response. For example, high radiation doses-per-fraction can induce Trex1 activity and hamper immune activation, but whether this occurs at clinically relevant radiotherapy (RT) fraction-sizes in breast cancer is unknown [2]. The presence of tumour infiltrating lymphocytes (TILs) confers a survival benefit in certain types of breast cancer (triple-negative, HER-2 positive), and predicts response to DNA-damaging agents, yet changes during RT are poorly described [3, 4]. Despite the emergence of numerous therapeutic agents targeting immune effector mechanisms, current radio-immunotherapy clinical trials are based on empiricism rather than an in-depth understanding of mechanisms underpinning the effects of radiation dose on immune-responsiveness in the clinical setting [5, 6, 7].

Neo-adjuvant RT provides a fantastic opportunity to study the effect of radiation directly on breast tumour and normal tissues irradiated in situ. The recently completed PRADA/Trans-PRADA trial (NCT02771938) successfully established the safety of neo-adjuvant RT prior to surgery in the radical/curative setting in high-risk breast cancer patients. Patient material from this study will provide novel radiobiological insights that have thus far been lacking. This information will be essential to guide the optimal timing and type of radio-immunotherapy combination therapy, as well as identifying

predictive biomarkers to assist in selecting which patients with high-risk breast cancer are most likely to benefit.

PROJECT AIMS

- To investigate the mechanisms linking DDRs and immunogenic cell death in irradiated breast cancers
- To characterise changes in the immune infiltrate pre- and post-radiotherapy using digital computational tools and multiplexed imaging platforms
- To elucidate changes in T-cell receptor repertoire in tumour & blood and correlate this with tumour response
- To correlate radiation-induced extra-cellular matrix remodeling with immune landscape and clinical response
- To identify novel biomarkers for tumour response/resistance to RT for personalised therapies

RESEARCH PROPOSAL

Patients (n=33) with high risk, non-metastatic breast cancer completed pre-operative loco-regional RT (40-42Gy in 15-16 daily doses) following primary systemic treatment, prior to mastectomy and immediate autologous breast reconstruction as part of the PRADA/Trans-PRADA clinical trial. Formalin-fixed, paraffin-embedded tissue has been obtained from the primary tumour: (1) at diagnosis (2) after fraction 1 of RT (3) after the final fraction of RT and (4) at mastectomy. Blood samples have been banked prior to, at the end of RT and at the time of surgery. Diagnostic and surgical tumour samples from control patients who did not have pre-operative RT have also been collected for comparison. Long-term clinical outcomes are available for all patients.

This unique dataset will, for the first time, allow a comprehensive analysis of radiation-induced immune changes in vivo correlated with tumour response. Following RNA extraction, expression of DDR and immune-activating genes will be analysed using Nanostring and validated using RNAseq. Following manual/automated scoring of TILs, the immune infiltrate will be further characterised using Vectra®3/CODEX quantitative pathology imaging [8, 9]. Longitudinal changes in T-cell receptor repertoire will be studied in tumour and blood and correlated with clinical responses. ECM remodeling pre- and post-RT will be studied using the TWOMBLI software [10]. Appropriate cellular and immune-competent animal models may be used for mechanistic studies, including responses to different RT dose-fractionation regimens. This information will guide rational combinations of RT with immunotherapy in future trials for high-risk breast cancer patients.

This work exploits the strong collaborative links existing between the Somaiah, Yuan and Melcher teams at ICR, including expertise within the Centre for Translational Immunotherapy at ICR and collaborators at the Crick Institute.

LITERATURE REFERENCES

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2. Vanpouille-Box C, Alard A, Aryankalayil MJ, et al. DNA exonuclease trex1 regulates radiotherapy-induced tumour immunogenicity. *Nat Commun* 2017;8:15618.
3. Denkert C, von Minckwitz G, Darb-Esfahani S, Lederer B, Heppner BI, Weber KE, et al. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. Lancet Oncol. 2018;19(1):40-50.
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8. De Angelis C, Nagi C, Hoyt CC, Liu L, Roman K, Wang C, Zheng Y, Veeraraghavan J, Sethunath V, Nuciforo P, Wang T. Evaluation of the Predictive Role of Tumor Immune Infiltrate in Patients with HER2-Positive Breast Cancer Treated with Neoadjuvant Anti-HER2 Therapy without Chemotherapy. Clinical Cancer Research. 2020 Feb 1;26(3):738-45.
9. Yuan J, Dakshinamoorthy G, Kim J, Mistry S, Nikulina N, Bashier R, Hempel C, Gallina ME, Kennedy-Darling J, Yuan J. Highly multiplexed single-cell spatial analysis of FFPE tumor tissues using CODEX®. Journal For Immunotherapy of Cancer 2019 Nov 6 (Vol. 7).
10. Wershof E, Barry DJ, Jenkins RP, Rullan A, Wilkins A, Roxanis I, Anderson KI, Park D, Bates PA, Sahai E. A FIJI Macro for quantifying pattern in extracellular matrix. bioRxiv. 2019 Jan 1:867507.

CANDIDATE PROFILE

Note: the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1)

Pre-requisite qualifications of applicants	First class or upper second class BSc Honours/MSc or equivalent in biological sciences or computational biology. Good communication and presentation skills
Intended learning outcomes Please provide a bullet point list (maximum of seven) of the knowledge and skills you expect the student to have attained on completion of the project.	• Develop an in-depth understanding of the DDR and immune crosstalk in relation to radiation in breast cancer • Understand the effects of radiation on the tumour, tumour immune microenvironment and extracellular matrix • Gain expertise in basic and advanced molecular pathology, including immunohistochemistry and next-generation sequencing • Gain experience in quantitative computational pathology with multiplex platforms such as VECTRA and CODEX • Scientific writing, presenting, team working and communication skills

ADVERTISING DETAILS	
Project suitable for a student with a background in	☒ Biological Sciences ☐ Physics or Engineering ☐ Chemistry ☐ Maths, Statistics or Epidemiology ☐ Computer Science ☒ Other (provide details) Bioinformatics, computational sciences
Keywords	**1.** Radiation-induced immune responses
	2. DNA damage response and immune cross-talk
	3. Breast cancer immune microenvironment
	4. Radio-immunotherapy in breast cancer
	5. Radiation and the extracellular matrix
	6. Digital computational tools for multiplex imaging